

Ascorbate Oxidase Inhibition in Dough by Fluoride Ion and Its Effect upon Dough Rheology

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ABSTRACT

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The effect of L-ascorbic acid (AA) on the rheological properties of bread dough can be measured by a simple spread test. The effect supposedly depends upon the oxidation of AA to dehydroascorbic acid (DHAA), which then behaves similarly to other oxidative improvers. Because fluoride ion is a potent inhibitor of the enzymatic oxidation of AA to DHAA, doughs containing AA and NaF were evaluated by the spread test

in comparison to doughs containing NaCl in place of NaF (Cl^- is a weak inhibitor of AA oxidation). The reduction in the spread ratio that characterizes the effect of AA on dough was only slightly diminished by the addition of F^- . The results indicated that the capacity of dough to oxidize AA to DHAA greatly exceeds that which is needed for the rheological effects of AA to be expressed.

A small amount of added L-ascorbic acid (AA) is often used as an improving agent in both conventional and short-time bread doughs. (Dahle and Murthy 1970, Nicolas et al 1980, Cherdkiatgumchai and Grant 1986). The mode of AA action in either type of dough system has been the object of considerable study and several hypotheses have been postulated, but the interpretation of the available data remains inconclusive (Cherdkiatgumchai and Grant 1986). Elkassabany and Hoseney (1980) reported that the factor(s) responsible for the high spread ratio in untreated doughs exist in the water-soluble fraction. This observation is consistent with Ewart's hypothesis (1985) on the "end blocking effect" of low molecular weight thiols upon desirable disulfide interchange reactions during dough development. A recent, new and different hypothesis attributes the effect of AA to the formation of intermolecular condensation products between the amino groups of flour proteins and dehydroascorbic acid (DHAA), the oxidation product of AA. (Pfeilsticker and Marx 1986).

Previous work has shown that AA disappears quite rapidly when added in small amounts to flour-water systems (Grant 1974) because of its oxidation to DHAA (Elkassabany et al 1980). Most of the oxidation is mediated by a specific AA-oxidase (or set of isozymes) although other oxidative enzymes, particularly lipoxigenase, appear to contribute indirectly (Cherdkiatgumchai and Grant 1986).

The purpose of the present work was to investigate whether or not the effectiveness of AA as an improving agent was limited in any way by the inherent capacity of dough to oxidize AA to DHAA. The experiments performed were based on the earlier observation that fluoride ion is a potent inhibitor of AA oxidation in flour-water systems (Grant and Sood 1980). If the capacity of

dough to oxidize AA is a limiting factor upon the rheological response of dough to AA supplementation, then the expected effect of including fluoride ion in the system would be to further limit that response.

MATERIALS AND METHODS

Samples of untreated flour milled from Canadian red spring wheat were obtained from a local commercial mill. Freshly milled flour was spread on the lab bench for several days to reduce the moisture content, mixed thoroughly, and stored at room temperature in metal cans. Flour A contained 9.6% moisture, 0.37% ash and 11.7% protein ($\text{N} \times 5.7$). Flour B contained 9.7% moisture, 0.49% ash, and 12.6% protein (as-is basis).

AA was purchased from Aldrich Chemical Co. DHAA was prepared from AA in aqueous solution by oxidation with I_3^- according to the United States Pharmacopeia (1970) (1.2 g of I_2 and 4.0 g of KI in 100 ml of H_2O). Titration of an AA solution with I_3^- was taken just past the end point (to yellow I_2 color) after which a few drops of dilute AA solution were added to dispel the yellow color to ensure that the DHAA solution contained no I_3^- . Both AA and DHAA solutions were freshly prepared just prior to their use.

All other chemicals were reagent grade.

Unyeasted doughs were prepared from 100 g of flour in a Morse model 17 LT Mixer (Denver, CO) with sufficient water to yield doughs with optimum or near optimum consistency. Water absorption was measured to the nearest 0.10 ml. The additives, AA, DHAA, NaCl, NaF, or combinations thereof, when included in the dough, were dissolved in the water of absorption.

The rheological behavior of the dough samples was evaluated by the spread test described by Hoseney et al (1979). After mixing for the desired time (usually 2.50 min) the dough was hand molded into a ball and placed in a fermentation cabinet for 60 min at $30 \pm 1^\circ\text{C}$ and 90% or greater relative humidity. The dough was again hand molded into a spherical ball (molding time 60 sec) and carefully placed on a glass plate and returned to the fermentation cabinet for a further 60 min. Immediately thereafter the height and width of the dough ball was measured with calipers to the nearest

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0.1 mm. The width was measured at four locations, rotating the glass plate 45° between each measurement. The spread ratio was calculated as the average width divided by the maximum height.

The disappearance of AA from doughs was measured using the modified indophenol-xylene extraction method (AACC 1983, Grant 1974). After mixing as described above, the dough was divided into three, approximately equal pieces (approximately 50 g), and each piece was weighed to the nearest 0.1 g. Dough pieces were held in the 30°C fermentation cabinet. At the desired time after the start of mixing, the dough piece was dispersed in a Waring Blender with 100 ml of metaphosphoric acid solution (MPA) to give a final concentration of 3% MPA. Acidification with MPA stopped the enzymatic oxidation of AA. The blender was operated for 30 sec, scraped down with a rubber spatula, operated for 30 sec more, scraped again, and operated for another 60 sec to give a total dispersion time of 120 sec. The suspension was centrifuged for 15 min at 15,000×g and appropriate aliquots of the supernatant were used for AA analysis. Two controls were run with each experiment. A zero-time control was performed in which a dough was prepared without AA but otherwise identical to the sample. An amount of AA equal to that added to the dough sample was added to the zero-time control sample at the time of dispersion with MPA solution. A blank control was also performed on a dough piece identical to the sample dough except that AA was omitted. After correcting the data for the small difference in weight of the dough pieces, the difference in absorbance between the blank control and the sample was directly proportional to the AA remaining in the sample, whereas the difference between the blank control and the zero-time control was directly proportional to the amount of AA originally added to the sample. This procedure for calculating the amount of AA that had disappeared from the sample dough provided an automatic correction for the small amount of dye-reducing substances that were endogenous to the flour.

RESULTS AND DISCUSSION

Preliminary experiments were conducted to investigate the effects of several variables on the spread ratio. The length of the dough mixing period was an important factor, with longer times associated with lower spread ratios (Fig. 1). There was no optimal mixing time when the spread ratio reached a minimum value, yet it was apparent from the appearance and feel of the dough that beyond 5 min, overmixing occurred. Thus, it is likely that for overmixed doughs there will be a poor correlation between the spread ratio and baking performance. Consequently the relationship between mixing time, baking performance, and spread ratio is in need of further investigation. However, these results clearly indicate that valid comparisons of spread ratio data

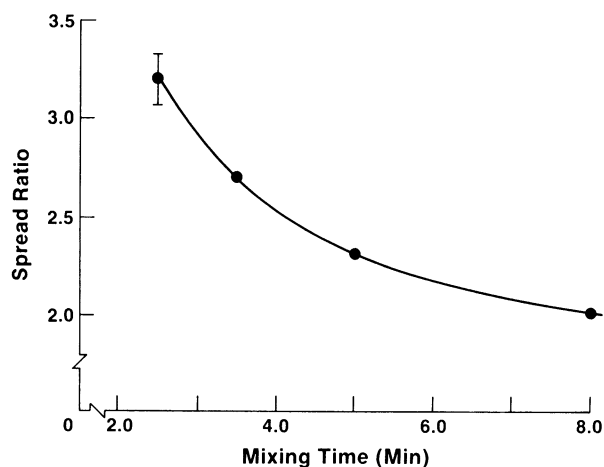


Fig. 1. Effect of mixing time on the spread ratio of dough (Flour A untreated, unyeasted dough, 1.0% NaCl, 56% absorption). The error bar represents one standard deviation.

depend upon close control of mixing times. From the handling properties of the dough, it was judged that optimum mixing was achieved in 2.50 min, and therefore a constant mixing time of 2.50 min was adopted for most experiments.

Increasing the amount of water resulted in softer doughs, as expected, although a 1% change in absorption resulted in a relatively small change in the spread ratio. Data are presented in Figure 2. In any event, the absorption could be controlled to a level of precision that considerably exceeded the precision of the spread ratio data.

Salt has a well-known effect upon dough rheology. Doughs containing 1.0% NaCl (flour basis) are stiffer and less sticky than doughs made without salt. Doughs containing the same molar concentration of NaF as 1.0% NaCl (i.e., 0.72% NaF) had very similar handling properties. In the absence of any added AA, the spread ratio for doughs prepared from flour A with 0.72% NaF averaged 3.14 ± 0.07 , which was essentially identical to the value of 3.19 ± 0.08 with 1.0% NaCl (averages of six or more replicates \pm standard deviation, 56% absorption, 2.5-min mixing time).

Experiments with flour A in which 100 ppm of AA was added to a 1.0% NaCl dough resulted in a $33 \pm 3\%$ decrease (four replicates) in the spread ratio compared to control doughs containing no AA. With 100 ppm of added AA in a 0.72% NaF dough, the spread ratio averaged $30 \pm 4\%$ (four replicates) less than the corresponding control. With only 5.0 ppm of added AA, the spread ratio was still $13 \pm 4\%$ (four replicates) less than the control in a 1.0% NaCl dough. The corresponding decrease in a 0.72% NaF dough was $8 \pm 6\%$ (six replicates). It is noteworthy that, in this work, only 5 ppm of added AA had a significant rheological effect upon the 1.0% NaCl dough. An earlier report indicated no detectable effect of added AA at this low level (Elkassabany and Hosenev 1980).

Within the limits of the precision of the data, these preliminary experiments indicated that the inhibition of AA oxidation by 0.72% NaF had no significant effect upon dough rheology. However, these data also suggested that if better data could be obtained it might be possible to demonstrate such an effect at low levels of added AA.

It was shown previously that NaF at 0.72% w/v caused 95% or greater inhibition in the rate of AA oxidation in flour-water suspensions (Grant and Sood 1980). At this stage of the investigation, no data were available on the inhibitory effect of NaF on AA oxidation in dough. However, the earlier work showed that salt inhibition of AA oxidation is greater in dilute flour-water suspensions than in more concentrated suspensions. Thus, it was assumed that 0.72% NaF would be less inhibitory in doughs than it was in flour-water suspensions. Therefore, further preliminary experiments were performed in which NaCl and NaF concentrations were both increased to 4.0 and 2.0%, w/v,

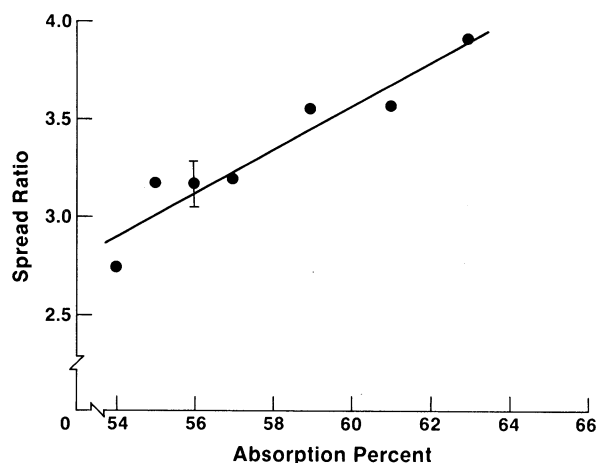


Fig. 2. Effect of water absorption on the spread ratio of dough (Flour A untreated, unyeasted dough, 1.0% NaCl, 2.50-min mix time). The error bar represents one standard deviation.

respectively. It was anticipated that a higher NaF concentration might inhibit AA oxidation so that the reduction in the spread ratio by AA would be less for NaF doughs than it was for NaCl doughs. The effects of added DHAA upon both NaF and NaCl doughs were also compared. In the 4.0% NaCl dough, 100 ppm of added AA resulted in a spread ratio of 2.27 ± 0.08 (flour A, 58% absorption, 2.5 min mixture, 6 replicates) compared to a ratio of 2.93 ± 0.10 (15 replicates) in the control, a decrease of $23 \pm 5\%$ while 100 ppm of added DHAA resulted in a spread ratio of 1.99 ± 0.05 (four replicates) a decrease of $32 \pm 4\%$. The corresponding effects upon 2.0% NaF doughs were decreases from 2.31 ± 0.09 (14 replicates) in the control to 2.10 ± 0.10 (five replicates) with 100 ppm of AA and to 1.99 ± 0.04 (four replicates) with 100 ppm of DHAA, for percentage decreases of only 8 ± 8 and $14 \pm 5\%$, respectively.

Thus, at these higher levels of salt, NaF had a much more pronounced dough stiffening effect than NaCl, especially when one takes into account the fact that 2.0% NaF is a lower molar concentration than 4.0% NaCl. The different effects of NaF and NaCl confound the interpretation of these data and cause some reservations about the earlier conclusion that 1.0% NaCl and 0.72% NaF had similar rheological effects.

The fact that 100 ppm of AA reduced the spread ratio of a 4% NaCl dough by only two thirds as much as 100 ppm of DHAA suggests that at this level of salt, chloride ion may be inhibiting the oxidation of AA enough to reduce the rheological response to AA treatment.

The inhibition of AA oxidation by F^- ion was investigated by comparing the rate of AA disappearance from unyeasted dough containing added NaF to that of a similar dough containing no added salt of any kind. Data are presented in Figure 3. The results showed differences between the two flours that were tested. After mixing for 2.5 min without any added salt, doughs made with flour A supplemented with 100 ppm of AA showed complete disappearance of the AA within 20 min. In contrast, in doughs that were prepared identically from flour B, the same level of added AA was approximately one-half oxidized within 5 min from the start of mixing, but very little further oxidation occurred as the dough rested during the next 85 min. These data may be compared to those reported by Carter and Pace (1965) who determined that oxidation of AA was rapid during mixing of unyeasted dough, with only 22% remaining after 5 min of mixing. However, these workers also observed that with only 1 min of mixing followed by a 90-min rest period, 50% of the added AA remained.

The oxidation of AA requires O_2 , and this reaction is only one of several that are competing for the available O_2 in dough. In dough made from flour B, it appeared that the O_2 that was incorporated during mixing became depleted early in the dough resting period. Thus AA oxidation slowed down markedly. In contrast, in dough from flour A that had a higher initial rate of AA oxidation, it

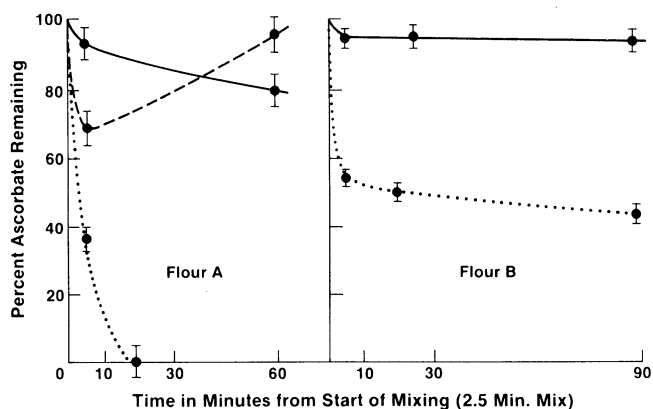


Fig. 3. Inhibition of ascorbic acid oxidation in dough by 0.72% NaF: — 100 ppm ascorbic acid (AA), 0.72% NaF; --- 20 ppm AA, 0.72% NaF; ... 100 ppm AA, flour-water dough (no NaF). The error bars each represent one standard deviation.

appeared that enough O_2 was incorporated to oxidize all the added AA.

It is well known that doughs are tolerant of added AA in excess of the minimum amount required to produce an optimum response. With most other improvers such as $KBrO_3$, an excess produces a detrimental effect. Except at very low substrate concentrations, the kinetics of AA oxidation in flour-water suspensions is zero order with regard to AA concentration, and the rate-limiting factor appears to be the concentration of endogenous ascorbate oxidase (Grant 1974). Therefore, a tolerance to excess AA would be the natural consequence of incomplete oxidation to DHAA because of the combined effects of relatively low ascorbate oxidase activity and depletion of available O_2 after dough mixing had stopped.

In the presence of 0.72% NaF, with both flour samples, the rate of AA oxidation at the 100 ppm level was reduced to approximately 10% of that observed in its absence. In doughs from flour A containing the same level of NaF but only 20 ppm of AA, approximately 30% was oxidized within 5 min, and thereafter it appeared that most of the resulting DHAA was reduced back to AA. In doughs made from flour A, containing 0.72% NaF and either 30 or 100 ppm of added AA, the absolute rate of AA oxidation as averaged over the first 5 min from the start of mixing, was approximately 1.0 ppm per min in the presence of 0.72% NaF. The initial rate of AA oxidation again appears to be independent of AA concentration, even in doughs where oxidation was inhibited by fluoride ion.

In the 0.72% NaF dough, which initially contained 30 ppm of added AA, there was an apparent reduction of DHAA back to AA at a slow average rate of approximately 0.1 ppm per min, which occurred after the O_2 had become depleted. This observation prompted further investigation. Unyeasted doughs were prepared with 0.72% NaF containing either no other additive or 100 ppm of DHAA. These doughs were analyzed immediately after mixing and again after a 90-min rest. The results in both cases indicated that substances analyzed as AA were appearing at the same slow rate as noted above. Because the result obtained was the same, with or without added DHAA, it is not possible to state conclusively that the substance was AA. Similar experiments with plain flour-water doughs without any additives did not reveal the slow appearance of any substances that were analyzed as AA. Thus, the appearance of such a substance was dependant upon the presence of NaF. As a result of these experiments, it may be that the data in Figure 3 slightly underestimate the extent of AA oxidation in the presence of 0.72% NaF.

In summary, inhibition of AA oxidation by fluoride ion in

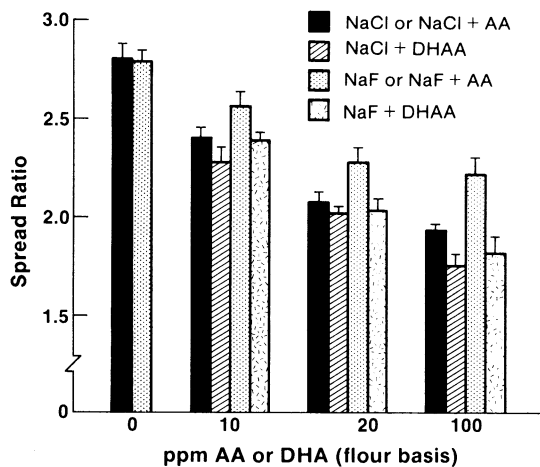


Fig. 4. Effects of ascorbic acid, dehydroascorbic acid, NaF, and NaCl on the spread ratio of doughs (Flour B, unyeasted doughs, 1.0% NaCl or 0.72% NaF, 63% absorption, 2.50-min mix time). Each bar represents the average of at least four and usually six or more replicates. Symbols at the top of each bar represent one standard deviation.

doughs was slightly less than that observed previously in flour-water suspensions. Nevertheless, there was marked inhibition.

Further inhibition studies were performed in which the NaF concentration was increased to 2.0%. The extent of inhibition of AA oxidation was not significantly higher than that observed in doughs with 0.72% NaF. This result suggests that the AA oxidation that did occur in the presence of either 0.72 or 2.0% NaF may have been nonenzymatic. The free radicals that are produced during dough mixing (Sidhu et al 1980) would be expected to catalyze some nonenzymatic oxidation of AA.

The comparative effects of 1.0% NaCl and 0.72% NaF on the rheology of doughs as measured by the spread test were further examined at several levels of added AA or DHAA using flour B. The results are shown in Figure 4. As the levels of either AA or DHAA increased, there were progressive decreases in the spread ratios of both NaCl and NaF doughs. Unlike the earlier results with flour A, the spread ratios were significantly higher for doughs containing NaF than they were for those containing NaCl at all levels of added AA that were tested. In contrast, at all levels of added DHAA differences between doughs containing NaF and NaCl were small, and at the 20 and 100 ppm levels these differences were not significant. The average spread ratio of the NaF doughs containing 100 ppm of AA was only slightly less than that of the NaCl doughs containing only 10 ppm. This agrees well with the result expected on the basis of the extent of NaF inhibition found for doughs made from this flour.

The rheological response of 1.0% NaCl doughs to added AA increased with increasing AA content at low levels of added AA, but above 50 ppm the incremental response became negligible. Therefore, it might be expected that the relative effect of fluoride upon dough rheology would be more pronounced at lower levels of added AA. This was not the case. Rather, at all levels of added AA that were tested, the decrease in the spread ratio in the presence of NaF was approximately 60% of that observed in the presence of NaCl. The apparent explanation for this result is that fluoride was less effective as an inhibitor of AA oxidation at lower AA concentrations. This explanation is supported by the data for flour A in Figure 3.

These data demonstrate that the inhibition of AA oxidation by fluoride ion does decrease the effect of AA on dough rheology for this one flour sample. However, the magnitude of the effect on dough rheology is considerably less than the effect on AA oxidation.

It was also observed with NaCl doughs that spread ratios were consistently lower for doughs containing DHAA than they were for doughs containing the same level of AA particularly at the 100 ppm level. This agrees with the earlier observation that AA in dough made from this flour was not completely oxidized, presumably because the system became depleted in oxygen.

CONCLUSIONS

Two untreated flour samples, both milled commercially from Canadian red spring wheat, differed with respect to both the extent of AA oxidation in flour-water doughs and the combined effects of NaF and AA on dough rheology. Doughs prepared with water from the first flour sample had the capacity to completely oxidize 100 ppm of added AA within 20 min. NaF at 0.72% inhibited this oxidation by approximately 90% but did not significantly influence the rheological effects of AA on the dough. Under the same conditions, water doughs prepared from the second flour sample oxidized AA somewhat more slowly during the first 5 min, and only about one half of the added AA was oxidized before the reaction stopped, possibly from lack of oxygen. NaF at 0.72%

again inhibited this oxidation by approximately 90%, but also decreased the rheological effect of added AA by a relatively small but significant amount.

Because simple salts alone affect dough rheology, the effects of NaF were evaluated in comparison to the same or similar molar concentrations of NaCl, which was known to have a very small inhibitory effect on AA oxidation. Most of the experiments were performed at low salt concentrations, where NaF alone had a very similar rheological effect to that of NaCl. However, at higher concentrations, NaF had a considerably greater dough stiffening effect. This difference introduced an element of uncertainty into the interpretation of the data.

In summary, NaF had either a small or a negligible influence on the rheological response of dough to AA supplementation despite the substantial inhibition that it caused toward AA oxidation. The overall conclusion was that the rheological response of a dough to AA supplementation will rarely be limited by a low capacity of the flour to enzymatically oxidize AA to DHAA.

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